International Journal of Oral and Craniofacial Science

Harleen Soni* and Manasi Vasavada

Department of Pedodontics and Preventive Dentistry, Manu bhai Patel Dental College, Near Vishwajoti Ashram, Munjmahuda, Vadodara, Gujarat, India

Dates: Received: 07 May, 2015; **Accepted:** 03 June, 2015; **Published:** 05 June, 2015

***Corresponding author:** Harleen Kaur Soni, Senior Lecturer, Department of Pedodontics and Preventive Dentistry, Manubhai Patel Dental College, India; Tel: + 91- 9687638664; E-mail: drharleen08@gmail.com

www.peertechz.com

ISSN: 2455-4634

Keywords: Dental caries; Polymerase chain reaction; Streptococcus *mutans*; Streptococcus *sorbinus*

Research Article

Distribution of *S. mutans* and *S. sorbinus* in Caries Active and Caries Free Children by PCR Approach

Abstract

Background: Streptococcus *mutans* (*S. mutans*) and Streptococcus sorbinus (*S. sorbinus*) have been considered to be the most important micro-organisms associated with dental caries. Therefore, purpose of this study is to detect and correlate the presence of *S. mutans and S. sobrinus* in the dental plaque of caries free and caries active children, by using Polymerase chain reaction (PCR) method.

Materials and Methods: Twenty patients aged between ages 4-8 years were included in the study. The subjects were divided in two groups: Group A consisting of ten children with early childhood caries and Group B consisting of ten caries-free children. Dental examinations were performed using a plane dental mirror and explorer. Plaque sample was collected from the cervical margin of the teeth by using explorer. PCR testing was performed for *S. mutans* and *S. sorbinus*. The data was analyzed by using SPSS software. The caries score data was analyzed among groups by applying Fischer's exact test. The P value <0.05 was considered as significant.

Results: *S. mutans* was present in all patients, whether they were caries active or caries free. There was no statistically significant difference in the presence of *S. sorbinus* in caries active patients as compared to caries free patients. It was also noticed that *S. sorbinus* was found more in male patients as compared to females, the difference being statistically non-significant.

Conclusion: Children harbouring both *S. mutans* and *S. sorbinus* had a higher incidence of dental caries as compared to the presence of either organism alone.

Introduction

Dental caries is one of the most common chronic infectious disease of childhood, caused by the interaction of bacteria, mainly Streptococcus *mutans*, and sugary foods on tooth enamel. These bacteria break down sugars for energy, causing an acidic environment in the mouth and result in demineralization of the enamel of the teeth and dental caries [1]. Dental caries in infants and toddlers is now collectively known as Early childhood caries (ECC). Early childhood caries results in a considerable direct burden of pain and suffering as well as poorer general health of the children. Despite efforts in restorative therapy, children who experience ECC continue to be at higher risk for new lesions in both the primary and the permanent dentition [2]. Understanding the role of specific bacterial species and subspecies is important for creating a complete model of caries aetiology.

There are three major hypothesis for the aetiology of dental caries: the specific plaque hypothesis, the nonspecific plaque hypothesis and the ecological plaque hypothesis [3-5]. The specific plaque hypothesis has proposed that only a few specific species, such as Streptococcus *mutans* (*S. mutans*) and Streptococcus sorbinus (*S. sorbinus*), are actively involved in the disease. According to the nonspecific plaque hypothesis, dental caries is the outcome of the overall activity of the total plaque micro flora, which is comprised of many bacterial species. The ecological plaque hypothesis suggests that caries is a result of a shift in the balance of the resident micro flora driven by changes in local environmental conditions [4].

Among the oral bacteria, *Mutans* streptococci have been implicated as a major cariogenic bacteria. The degree of colonization of these organisms correlates with the prevalence of dental caries in children and experimental animals.

Mutans streptococci is divided into seven species: Streptococcus *mutans*, Streptococcus sobrinus, Streptococcus downei, Streptococcus rattus, Streptococcus cricetus, Streptococcus ferus, and Streptococcus macacae. Among these *S. mutans and S. sobrinus* are strongly associated with human dental caries [6]. Epidemiological studies have reported that *S. mutans* is more prevalent than *S. sorbinus* in the oral cavity, but have also shown that the prevalence of *S. sorbinus* is more closely associated with a high caries experience [7].

Molecular methods for bacterial identification and enumeration now make it more precise to study the microbiota associated with caries. DNA sequence-based assays can be used to identify closely related species that are difficult to differentiate by traditional, culture-based approaches [8]. Various methods have been used for detection, including biochemical tests, immunological tests, DNA probes, and Polymerase Chain Reaction (PCR). Among these, the PCR method is currently being applied to the detection of putative pathogens and the identification of human cariogenic bacteria because it is rapid, sensitive, and simple.

The purpose of this study is to detect and correlate the presence of *S. mutans and S. sobrinus* in the dental plaque of caries free and caries active pre-school children, by using PCR method.

Materials and Methods

The present study was conducted in the Department of Pedodontics and Preventive Dentistry at Manu bhai Patel Dental College, Vadodara.

Ethical approval

Ethical clearance was taken from the University Ethical Committee before conducting the study. Full detailed treatment and benefits were explained to the parents of the children and written informed consent for the treatment was taken from the parent/guardian prior to participation of subjects in the study.

Twenty patients aged between ages 4-8 years were included in the study. The subjects were divided in two groups: Group A consisting of ten children (five males and five females) with early childhood caries and Group B (five males and five females) consisting of ten caries-free children. The following patients were included in the study: (I) Children having more than 6 carious lesions were included in the study for Group A. The World Health Organization (WHO) caries diagnostic criteria was used for determining the dmft (decayed, missing, filled) index. (II) Children having no existing caries were included for Group B. Patients who had any of the following criteria were excluded from the study: (I) Children who were on antibiotics within the past 3 months (II) Children with any systemic diseases were excluded from the study (III) Children with existing restorations on any surface of tooth were excluded from the study and (IV) Medically compromised children.

Sampling

Dental examinations were performed using a plane dental mirror and explorer. Plaque sample was collected from the cervical margin of the teeth by using explorer. Each explorer was immediately placed in a tube containing sterile pH 7.0 phosphated-buffered saline, and frozen immediately at -20°C.

Isolation of DNA and PCR assays

DNA extraction from samples was performed using Merc DNA Saliva Extraction Kit according to the manufacturer's instructions: Plaque samples were first harvested by centrifugation at 1600 g for 20 min. The supernate were discarded and individual cell pellets were stored at -20°C until DNA isolation. The genomic DNA preparation from each plaque sample was obtained by a standard miniprep procedure, with the addition of an RNA ase treatment. PCR testing and amplification was performed as previously reported for: *S. mutans* and *S. sorbinus*. PCR amplification was performed in a reaction mixture (25 µl) consisting of PCR beads that contained an enzyme and the required reagents, 25 pmol of each primer and 20–50 ng of the template DNA solution in a thermal cycler. The reaction mixture was denatured at 95°C for 3 min followed by a series of amplifications: denaturation at 95°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min. The series was repeated for 26 cycles. The final cycle comprised 94°C for 1 min, 55°C for 1 min and 72°C for 5 min. After amplification, 15 µl of the PCR products were analyzed by electrophoresis on an agarose 1.2% gel. The newly synthesized DNA fragments were visualized under ultraviolet light at 302 nm after staining with ethidium bromide. The size of the PCR products was estimated from the electrophoretic migration of products relative to a 100-bp ladder. The data was analyzed by using SPSS. The caries score data was analyzed among groups by applying Fischer's exact test. The P value <0.05 was considered as significant.

Results

Table 1 shows the age and dmft values of the children included in the study.

Table 2 shows the distribution of various organisms between children according to their caries status. *S. mutans* was present in 100% of the subjects. *S. sorbinus* was present in increased number in the caries active group than the caries free group but the difference was not significant.

The Fischer's test values for the distribution of micro-organisms between children who were caries active and caries free was 0.45, which was not significant.

Discussion

Dental caries is an infectious disease in which members of the *Mutans* streptococci have been implicated as the etiological agents. Thereby, this study was undertaken to understand the association of *S. mutans* and *S. sorbinus* to the presence of dental caries in children. In the present study, dental plaque was used as a source for detection of cariogenic bacteria rather than bacteria from saliva. As the intention is to relate the presence of cariogenic bacteria and dental caries, using saliva as a source of the bacteria does not permit establishing an effective association. Although the presence of *S. mutans* is high in saliva, it is lower on the surface of enamel, where the bacteria actually manifests its capacity to produce acids leading to subsequent demineralization [9].

Table 1: Age Distribution and Dmft Index of Children.

Patient	1	2	3	4	5	6	7	8	9	10
Age	4	6	3	5	4	5	4	3	5	6
dmft	6	4	7	5	6	0	0	0	0	0

Table 2: Distribution of Various Organisms between Children According To Their Caries Status.

Sr No.	Species	Caries Active (n=10)	Caries Free (n=10)	P-corelation Cofficient
1.	+ve for S. Mutans	10 (100%)	10 (100%)	0
2.	+ve for S. sorbinus	6 (60%)	3 (30%)	0.18

In this study, supra gingival plaque samples were collected from the cervical margins of both caries and caries free tooth surfaces with the help of a sterile dental explorer. Various authors as Carmona et al. [9], Choi et al. [10] and Okada et al. [11], in their respective studies collected supra gingival plaque samples with the dental explorer for the detection of *S. mutans and S. sobrinus* from children without caries, with early childhood caries and severe early childhood caries.

The detection and identification of oral streptococci in the dental biofilm is considered to be an important step for the understanding of dental caries. Nearly all investigations into the microbial pathogenesis have been done by cultivation of bacteria. This conventional method of culturing the bacteria is more time consuming and is sometimes, inaccurate. It has also been reported that MS-bacitracin inhibits the growth of *S. sorbinus* to a greater extent than that of *S. mutans* on the agar medium, thereby providing inappropriate results [8]. However, the advanced molecular methods have revealed that the bacterial involvement in the development of dental caries is more complex than previously believed in the present study, PCR method was used for amplification of the gene sequences of *S. mutans and S. sobrinus*. According to Igarashi et al. [12], PCR is a rapid, more sensitive and simpler method for the detection of micro-organisms. They also reported that the conventional cultural methods used for the detection of micro-organisms in dental plaque takes a long time, nearly 1 week whereas PCR method reduces the time to 6-7 hours. PCR was used in a number of studies to detect micro-organisms and to evaluate their association with dental caries [13-16].

PCR technique requires the use of primers for specific organisms. Primers are short pieces of single-stranded DNA that are complementary to the target sequence. In our study, oligonucleotide primers used were GTFB- F5'- ACTACACTTTCGGGTGGCTTGG and GTFB- R5'- CAGTATAAGCGCCAGTTTCATC, designed to amplify a 517 bp (base pair)-DNA fragment of the gtf B gene sequence of Streptococcus *mutans*, and GTFI- F5'- GATAACTACCTGACAGCTGACT and GTFI- R5'- AAGCTGCCTTAAGGTAATCACT, designed to amplify a 712 bp (base pair)-DNA fragment of the gtf I gene sequence of Streptococcus sobrinus. The gtfB and gtfI genes of Streptococcus *mutans* and Streptococcus *sobrinus* respectively are suitable for designing PCR primers, as both genes express important virulence factors of the cariogenic bacteria and have nucleotide sequences specific to each species [17]. The same set of primers were used for the detection of *S. mutans and S. sobrinus* in studies done by Carmona et al. [8], Seki et al. [18] and Franco e Franco et al. [19] and they yielded substantial results.

In our study, the results showed that both *S. mutans and S. sobrinus* were present in 60% of caries active samples and 30% of caries free samples, which was almost double in caries active samples as compared to caries free samples. Similar results were seen in studies indicating the likelihood of the presence of both *mutans* streptococci to be three times more in caries active group as compared to caries free group [10,14,20].

In the present study, *S. mutans* was detected in all subjects, whether they were caries active or caries free. A study by Rodriguez et al. [21], on Mexican preschool children, showed the percentage of *S. mutans* isolation was 75% in caries active children and 60% in caries free children and another study by Choi et al. [10] also showed that *S. mutans* was detected 100% in children with dental caries and 80% in children without dental caries. Both these studies showed no statistically significant difference the studies conducted by Loesche and Straffon. [22] and Aas et al. [23], demonstrated that caries can occur in the absence of *S. mutans*. The results of these studies had similar results in which *mutans* was associated with both caries active and caries free children, contradicting its role in the initiation and progression of caries [24-26] In contrast, Carmona et al. [9], found that the frequency of *S. mutans* was 76% in subjects with caries and 24% in subjects without caries showing a statistically significant difference. Ge et al. [27], demonstrated that ECC is associated not only with increased levels of *S. mutans* but also due to elevated levels total streptococci in the mouth. *S. mutans* is a dominating species, widespread in populations with low caries prevalence, indicating that the ecological determinants of these bacteria are not necessarily associated with a caries promoting lifestyle [28]. Matee et al. [24] and Sullivan et al. [25], observed that the level of oral streptococci in the saliva of children cannot predict future caries. The importance of *S. mutans* in caries etiology has been well documented, but the growing recognition that the cariogenic potential may be determined by complex interactions in dental plaque biofilm rather than solely the virulence properties of a single organism [29] hence, there was no clear relationship between caries experience and the presence of *S. mutans*.

The present study also showed that *S. Sobrinus* was present in 60% of caries active group whereas 30% was present in caries free group and the difference was statistically significant. Carmona et al. [9], in their study reported that the frequency of *S. Sobrinus* was 81.9% in carious lesions and 18.1% in caries free surfaces with the difference being statistically significant. Choi et al. [10], showed in their study that *S. sobrinus* was detected less frequently than *S. mutans* in caries free children. They reported that *S. sobrinus* was detected in 43-60% in early childhood caries group and 8.6% of caries free group. Similarly, Rodriguez et al. showed that *S. sobrinus* was isolated more than twice as high in caries active children as compared with caries free individuals, this suggests that *S, sorbinus* was associated with active dental caries and children with caries experience [21]. Beighton et al. [30], however found no correlation between the caries experience and the salivary *S. sorbinus* level. It is important to consider that different strains of *Mutans* streptococci induce different levels of dental decay in animal models, probably because *S. sorbinus* produces acid more rapidly than *S. mutans* [31].

Conclusion

There was a different distribution of *mutans* streptococci between caries-free and caries-affected children. The presence of *S. sorbinus* was more closely related to the presence of dental caries in contrast to *S. mutans*. Children harbouring both *S. mutans* and *S. sorbinus* had a higher incidence of dental caries as compared to the presence of either organism alone.

References

1. Colak H, Dulgergil CT, Dalli M, Hamidi MM (2013) Early childhood caries update: a review of causes, diagnosis, and treatments. J Nat Sci Biol Med 4: 29–38.
2. Low W, Tan S, Schwartz S (1999) The effect of severe caries on the quality of life in young children. Pediatr. Dent 21: 325-326.
3. Loesch WJ (1992) The specific plaque hypothesis and the antimicrobial treatment of periodontal disease. Dent Update 19: 68-74.
4. Marsh PD (1994) Microbial ecology of dental plaque and its significance in health and disease. Adv Dent Res 8: 263-271.
5. Theiland E (1986) The non-specific theory in microbial etiology of the inflammatory periodontal disease. J Clin Periodontol 13: 905-911.
6. Loesche WJ (1986) Role of streptococcus mutans in human dental decay. Microbiol Rev 50: 353-380.
7. Hirose H, Hirose K, Isogai E. Miura H, Ueda I (1993) Close association between Streptococcus sorbinus in the saliva of young children and smooth-surface caries increment. Caries Res 27: 292-297.
8. de Soet JJ, van Dalen PJ, Pavicic MJAMP, de Graaff J (1990) Enumeration of mutans streptococci in clinical samples by using monoclonal antibodies. J Clin Microbiol 28: 2467-2472.
9. Carmona LE, Reyes N, González F (2011) Polymerase chain reaction for detection of Streptococcus mutans and Streptococcus sobrinus in dental plaque of children from Cartegena, Colombia. Colomb Med 42: 430-437.
10. Choi EJ, Lee SH, Kim YJ (2009) Quantitative real-time polymerase chain reaction for Streptococcus mutans and Streptococcus sobrinus in dental plaque samples and its association with early childhood caries. Int J Pediatr Dent 19: 141-147.
11. Okada M, Kawamura M, Oda Y, Yasuda R, Kojima T, et al. (2012) Caries prevalence associated with Streptococcus mutans and Streptococcus sobrinus in Japanese school children. Int J Paediatr Dent 22: 342–348.
12. Igarashi T, Yamamoto A, Goto N (2000) PCR for detection and identification of Streptococcus sobrinus. J Med Microbiol 49: 1069-1074.
13. Childers NK, Osgood RC, Hsu KL, Manmontri C, et al. (2011) Real-time quantitative polymerase chan reaction for enumeration of Streptococcus mutans from oral samples. Eur J Oral Sci 119: 447-454.
14. Okada M, Soda Y, Hayashi F, Doi T, Suzuki J, et al. (2002) PCR detection of Streptococcus Mutans and S. sorbinus in dental plaque samples from Japanese pre-school children. J Med Microbiol 51: 443-447.
15. Hata S, Hata H, Miyasawa-Hori H, Kudo A, Mayangi H (2006) Quantitative detection of Streptococcus mutans in the dental plaque of Japanese preschool children by real-time PCR. Lett Appl Microbiol 42: 127-131.
16. Yano A, Kaneko N, Ida H, Yamaguchi T, Hanada N (2002) Real-time PCR for the quantification of Streptococcus mutans. FEMS Microbiol Lett 21: 23-30.
17. Ono T, Hirota K, Nemoto K, Fernandez EJ, Ota F, et al. (1994) Detection of Streptococcus mutans by PCR amplification of spaP gene. J Med Microbiol 41: 231-235.
18. Seki M, Yamashita Y, Shibata H, Tsuda H, Maeno M (2006) Effect of mixed mutans streptococci colonization on caries development. Oral Microbiol Immunol 21: 47-52.
19. Franco e franco TCC, Amoroso P, Marin JM, Avila FA (2007) Detection of Streptococcus mutans and Streptococcus sobrinus in dental plaque samples from Brazilian preschool children by polymerase chain reaction. Braz Dent J 18: 329-333.
20. Nurelhuda NM, Al-Haroni M, Trovik TA, Bakken V (2010) Caries experience and quantification of Streptococcus mutans and Streptococcus sobrinus in saliva of Sudanese school children. Caries Res 44: 402-407.
21. Rodriguez J, Martinez R, Ferreyra B, Marin N, Solis A, et al. (2008) Distribution of Streptococcus mutans and Streptococcus sobrinus in saliva of Mexican preschool caries-free and caries-active children by microbial and molecular (PCR) methods assays. J Clin Pediatric Dent 32: 121-126.
22. Loesche WJ, Straffon LH (1979) longitudinal investigations of the role of Streptococcus mutans in human fissure decay. Infect Immun 26: 498-507.
23. Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen I, et al. (2008) Bacteria of dental caries in primary and permanent teeth in children and Young Adults. J. Clin Microbiol 46: 1407-1417.
24. Matee MI, Mikx FH, Maselle SY, Van Palenstein Helderman WH (1992) Mutans streptococci and lactobacilli in breast-fed children with rampant caries. Caries Res 26: 183-187.
25. Sullivan A, Borgstorm MK, Granath L, Nilsson G (1996) Number of mutans streptococci or lactobacilli in a total plaque sample does not explain the variation in caries better than the numbers in stimulated whole saliva. Community Dent Oral Epidemiol 24: 159-163.
26. Giacaman RA, Arneda E, Padilla C (2010) Association between biofilm-forming isolates of mutans streptococci and caries experience in adults. Arch Oral Biol 55: 550-554.
27. Ge Y, Caufield PW, Fisch GS, Li Y (2008) Streptococcus mutans and Streptococcus sanguinis colonization correlated with caries experience in children. Caries Res 42: 444–448.
28. Carlsson P, Gandour AI, Olsson B, Rickardsson B, Abbas K (1987) High prevalence of mutans streptococci in a population with extremely low prevalence of dental caries. Oral Microbiol Immunol 2:121-124.
29. Martinez-Martinez RE, Fujimara T, Patino-Martin N, Hoshino T, Wilson M, et al. (2012) Comparison of oral streptococci biofilm in caries-free and caries-affected pre-school Mexican children. Acta Odontol Latinoam 25: 27-32.
30. Beighton D, Manji F, Baelum V, Fejerskov O, Johnson NW, et al. (1989) Associations between salivary levels of Streptococcus mutans, Streptococcus sobrinus, Lactobacilli and caries experience in Kenyan adolescents. J Dent Res 68: 1242-1246.
31. de Soet JJ, Van Loveren C, Lammens AJ, Pavicic MJ, Homburg CH, et al. (1991) Differences in cariogenicity between fresh isolates of Streptococcus sorbinus and Streptococcus mutans. Caries Res 25: 116-122.

Citation: *Soni H, Vasavada M (2015) Distribution of S. mutans and S. sorbinus in Caries Active and Caries Free Children by PCR Approach. Int J Oral Craniofac Sci 1(1): 027-030.*